PATENT
Docket No.: 176/61442 (1196)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Miller et al.	Examiner: Sarae Bausch
Serial No.	:	10/541,044	
Cnfrm. No.	:	1984	Art Unit: 1634
Filed	:	January 2, 2004	
For	:	HYBRIDIZATION-BASED BIOSENSOR CONTAINING HAIRPIN PROBES AND USE THEREOF	

SECOND DECLARATION OF BENJAMIN L. MILLER UNDER 37 C.F.R. § 1.132

I, BENJAMIN L. MILLER, pursuant 37 C.F.R. §1.132, declare as follows:

1. I am an inventor of the above-identified application, and I previously signed a declaration that was presented to the U.S. Patent and Trademark Office ("PTO") on November 23, 2009. My credentials are identified in paragraphs 2-3 of that prior declaration.

2. I am presenting this declaration to address with particularity the objections raised by the PTO in the Advisory Action dated December 30, 2009.

3. I note in passing that the PTO appears to be confused about the reference to dithiols in applicants' previous arguments. Cass mentions the use of dithiols, but as acknowledged by the PTO in the prior office action at page 3, Cass does not teach the use of these dithiols in the manner as claimed, i.e., a mixture of spacer molecules and hairpin molecules at a ratio of 5:1 or greater. Nevertheless, it is Cass's use of dithiols that applicants referred to, not a limitation of the claims.

4. Claim 1 of the present application recites a process limitation in defining the resulting product. This process limitation concerns the "exposure of the fluorescence quenching surface to a mixture comprising a ratio of spacer molecule to first nucleic acid molecule of about 5:1 or greater." This process limitation is used to define the resulting product, because this process limitation is what allows the hairpin probes to fold and thereby afford reduced background signaling. The reduced background signaling is what allows the

"sensor chip [to exhibit] at least a 5-fold increase in fluorescent emissions intensity when the sensor chip is exposed to a target nucleic acid molecule that hybridizes specifically to the first nucleic acid molecule" as claimed.

5. Neither Cass, Herne, nor the combination thereof teach a process that can achieve the claimed product. That is because the processes of Herne and Cass will not result in a functional product that can achieve the limitations of the claimed product. The previously submitted experimental evidence confirms that the optimal process conditions of Herne, if used with a hairpin probe, such as that taught generally by Cass, will not result in a sensor chip that exhibits "at least a 5-fold increase in fluorescent emissions intensity when the sensor chip is exposed to a target nucleic acid molecule that hybridizes specifically to the first nucleic acid molecule" as claimed.

6. On page 4 of the Advisory Action, the PTO asserts that "Herne teaches the substrate has spacer molecules with a ratio of 5:1 and thus it would have been obvious to modify the sensor chip of Cass with the spacer molecules of Herne." This assertion of the PTO ignores the fact that one of skill in the art would be concerned with the process that Herne teaches for arriving at their schematically illustrated structure rather than the illustration *per se*. Thus, one of skill in the art would have utilized Herne's proclaimed optimal conditions to actually undertake the introduction of Herne's spacer molecules (MCH) in conjunction with hairpin probes of the type taught in Cass. A person of skill in the art would understand that the schematic illustration of Herne depicting a 5:1 ratio of spacer molecules to probe on the surface of the chip is nothing more than an illustration—it is not an indication that such a condition existed on the surface of the chip. In fact, one cannot *a priori* calculate how much hairpin probe is present on the chip in the Cass/Herne case.

7. On page 5 of the Advisory Action, the PTO asserts that "[t]he substrate of applicants has almost a 10:1 ratio of probe:target …while the substrate of Cass and Herne provided for by applicant is only a 2.5 fold difference…." Neither of these statements is true. The PTO has apparently confused the concentration used to prepare the chip (i.e., the process conditions) with the concentration used in the assay. There is no indication how much probe remains on the chip surface following the combined Cass/Herne process. However, whether using the process of the presently claimed invention or the sequential process of Herne, the

amount of probe remaining on the surface should be substantially lower than the amount of target used during the assay (i.e., there should be a large excess of target relative to probe). Thus, the different ratios cited by the PTO are insignificant.

8. At the bottom of page 5 and top of page 6 of the Advisory Action, the PTO asserts that "...the hairpin probe provided for in the declaration is not the hairpin probe disclosed by Cass. Cass exemplifies a 5' end with fluorescein and a 3' end biotin group attached to a linker." While the PTO is entirely correct in this regard, the reason for the use of a different hairpin probe is because the Cass probe (cited by the PTO) would not under any circumstances yield a functional chip that can achieve at least a 5-fold increase in fluorescent emissions intensity when the sensor chip is exposed to a target nucleic acid molecule that hybridizes specifically to the first nucleic acid molecule. The reason why this is true is because there is no way to attach a biotinylated DNA probe to a gold chip, unless one had some way of first attaching streptavidin to the chip. If streptavidin were used as a first attachment layer, its size would prevent the fluorophore from being brought in proximity to the metal film, and therefore one would have the weak streptavidin-based quenching described by Cass rather than the strong metal-based quenching described in the present application. Thus, the use of a different hairpin probe in combination with the Herne process steps afforded the best possible chance for the combination of Cass/Herne to yield a functional chip. Even so, as explained in my prior declaration, this was not enough to overcome the deficiencies associated with the chip preparation steps as taught by Cass/Herne. The combination of Cass/Herne would not afford a sensor chip as presently claimed.

9. On page 6 of the Advisory Action, the PTO concludes that the teachings of Herne are consistent with the teachings of the present application. I disagree with this statement, because while both Herne and the present invention discuss the use of thiols (Herne specifies MCH and the preferred thiol in the present application is mercaptopropanol), Herne's approach for the use of the MCH is *very* different for the use of the spacer molecule in the manner as claimed. As discussed above, Herne's sequential use of probe and then MCH is not at all like the simultaneous exposure recited in the claims. The significant difference is that the sequential approach taught by Herne will not afford a functional chip when using hairpin probes. The experimental results, using the *optimal* conditions of Herne, confirm as much. The

comparative data was offered to confirm that the probe and the process limitations of claim 1 afford a functional chip. Even discounting the comparative evidence due to the differences between the two sets of data, the evidence of record proves that the optimal conditions of Herne when combined with the use of a hairpin probe (that is otherwise capable of functioning properly), will not produce a functional chip.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: January 21, 2010

Benjamin L. Miller, Ph.D